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THE DENTO-GINGIVAL JUNCTION AS SEEN WITH LIGHT MICROSCOPY AND SCANNING ELECTRON MICROSCOPY

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Abstract

The purpose of this paper is to review the anatomical relationship of the Dento-Gingival Junction as seen in the human dentition. The junction is described under light microscopy and then reviewed as seen in the SEM with the authors' unpublished findings. The authors' material was derived from extracted human teeth with remaining marginal gingival tissue. The specimens were fixed with 2% glutaraldehyde in 0.15M sodium cacodylate buffer (pH 7.2) for 24 h. The specimens were then washed and freeze-fractured in Freon 113 using liquid nitrogen. Afterwards they were processed by freeze-drying or CPD methods, coated with gold, and placed in the scanning electron microscope (SEM) for viewing. These specimens demonstrated the presence of numerous Sharpey's fibers at the cemental surface. A large number of fibrils intermingled with the fibers to produce a dense mass of tissue. Junctional epithelium, with the adjacent homogeneous dental cuticle was demonstrated. Plaque deposits on the tooth surface extended to a cell-free zone. Morphological detail viewed with SEM and light microscopy are compared.

Introduction

The oral tissues that retain teeth in the socket are gingiva, attachment epithelium, periodontal ligament, the cementum and the alveolar bone proper of the alveolar process. The dento-gingival junction (DGJ) refers to that portion of the gingiva that extends from the gingival margin to the crest of the alveolar bone. This junction consists of the lamina propria and its epithelial covering, fibrous attachment to cementum (cemental fibers), attachment epithelium (junctional epithelium), and sulcus as demonstrated in Figure 1. The basic function of the DGJ is to produce a seal or barrier between the contents of the oral cavity and the periodontal ligament (Ramfjord et al. 1966, Garant and Mulvihill 1971a-b, Geisenheimer and Han 1971, Gavin 1972). Other functions include the maintenance of approximate tooth contact (Picton and Moss 1980, Moss and Picton 1967), tooth alignment (Glenwright 1970) and spread of occlusal forces throughout the arch (Picton 1962).

The purpose of this paper is to describe the DGJ as seen in light microscopy and compare this to observations obtained with the use of SEM. Knowledge of the DGJ is important in the study of the pathology and treatment of periodontal diseases.

Light Microscopy

The DGJ covering consists of oral and dental epithelia. The dental epithelia contain two morphological types: the sulcular and junctional epithelia. The former borders a space (the sulcus) with the tooth surface, either enamel or dentin. The junctional epithelium extends approximately 0.98 mm (range 1.35 - 0.71 mm with age) apical from the base of the sulcus to the cemento-enamel junction or to a location on the cementum when loss of connective tissue attachment occurs (Garguilo et al. 1961). In the facial and lingual areas, the oral epithelium is usually

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parakeratinized (McHugh 1964, Cleaton-Jones and Fleisch 1973, Cleaton-Jones et al. 1978) stratified squamous epithelium containing basal, prickle cell, granular and cornified layers (Listgarten 1964, Frithrob and Wersall 1965, Schroeder and Theilade 1966). The width of the keratinized surface of the gingiva can vary; therefore non-keratinized mucosa may cover part of the DGJ (Bowers 1963, Ainamo and Loe 1966, Lang and Loe 1972, Miyasato et al. 1977, Voigt et al. 1978, Tenenbaum and Tenenbaum 1986). The epithelial cells are highly organized to maintain attachment to each other with the presence of narrow intercellular spaces, desmosomes and tight junctions. The morphology of the epithelial cell attachments appear to compartmentalize this tissue and offer better resistance to all but small molecular substances. This epithelium also shows Langerhans cells and melanocytes (Schroeder 1969a). The Langerhans cells may be related to recognition of mitogen that may enter the epithelial structure (Walsh et al. 1986). The basal layer has repeated evaginations into the connective tissue developing ridges parallel to the gingival margin (Ooya and Tooya 1981). The ridges fuse at sites to form surface depressions, called stippling (Orban 1948). The epithelial-connective tissue interface consists of basal lamina with hemidesmosomes and attachment to the adjacent connective tissue.

At the interproximal area, the Dento-Gingival Junction extends from one tooth surface to the adjacent tooth surface with the lamina propria continuous between the two surfaces. The oral epithelia that covers this tissue is generally parakeratinized except directly apical to the contact of adjacent teeth. At this area, the type of epithelium may be non-keratinized squamous type (Kohl and Zander 1961, Kaplan 1977, Kaplan et al. 1977).

The gingival sulcus is the space between the gingival margin and the tooth, and extends to the junctional epithelium. In the adult, the histological depth of the sulcus is approximately 0.5 mm (Attstrom et al. 1975) but can be deeper (1.43 - 1.86 mm), (Ainamo and Loe 1966). Clinical depth of sulcus will vary with age, approximating 3 mm in the adult. (Smith 1982). Plaque usually extends to the base of the sulcus (Saglie et al. 1974, Saglie 1977) and contains inflammatory cells (Attstrom 1970, Garant and Mulvihill 1971a).

The sulcular surface is non-keratinized squamous epithelium and consists of superficial, spinous and basal layers (Gavin 1968, Garant and Mulvihill 1971b, Geisenheimer and Han 1971). The basal layers contain finger-like projections into the connective tissue as a result of increased

mitotic activity in possible response to irritation.

The junctional epithelium is non-keratinized and may vary in thickness from one to eighteen cells (Schroeder 1969b). An excellent review of the literature is presented by Schroeder and Listgarten (1971). The tissue contains prickle-like cells and consists of an internal (oral) and external (dental) attachment (Listgarten 1972) to connective tissue and tooth structure respectively (Schroeder 1969a, Yamaski et al. 1979). The attachments are similar to basement membrane consisting of a basal lamina system (Stallard et al. 1965) and hemidesmosome plaques (Listgarten 1966b, Weiss and Neiders 1970, Kobayski et al. 1976, Saglie et al. 1979, Marikova 1983). The attachment to the tooth may be mediated by a homogeneous membrane called the dental cuticle (Waerhaug 1956, Wertheimer and Fullmer 1962, Listgarten 1966b, Taylor and Campbell 1972, Listgarten 1976, Kobayaski et al. 1976, Kobayaski and Rose 1978, 1979, Newman 1980, Abbas et al. 1985). The basal cells adjacent to the connective tissue have a low mitotic rate (Garnick 1977). Daughter cells migrate diagonally to the tooth where they develop an external attachment to the tooth surface (Anderson and Stern 1966). The cells then migrate coronally toward the sulcus, and desquamate into the oral cavity (Schroeder and Munzel-Pedrazzali 1970). The apical cells of the junctional epithelium are prevented from migrating further on the tooth surface by dense (Sharpey) cemental fibers (Goldman 1944, 1951, Listgarten 1966b, Saglie et al. 1975a).

The lamina propria of the gingiva consists of mostly interlacing collagen fiber bundles which vary in size (Smuklar and Dreyer 1968). A few elastin-like fibers are located near blood vessels (Lopez et al. 1976, Porter et al. 1977). Also, specialized fibers called oxytalan fibers are present (Shackleford 1972, Kvam 1972, Sloan 1978, Sims 1983). The principal bundle of fibers consists of the dentogingival, alveologingival, circular and longitudinal groups. The dentogingival fibers are cemental fibers that originate from the cementum and extend into the lamina propria to interlace with other fibers as shown in Figure 2 (Page et al. 1974, Garnick and Walton 1984). These fibers attach to the cementum that extends from the base of the junctional epithelium to the level of the alveolar crest, a distance of approximately 1 mm (Garguilo et al. 1961). The alveologingival fibers originate from the crest of the alveolar bone and insert into the fibers of the lamina propria. The circular group are fibers that encircle the teeth and the longitudinal group extend throughout the arch on the buccal or

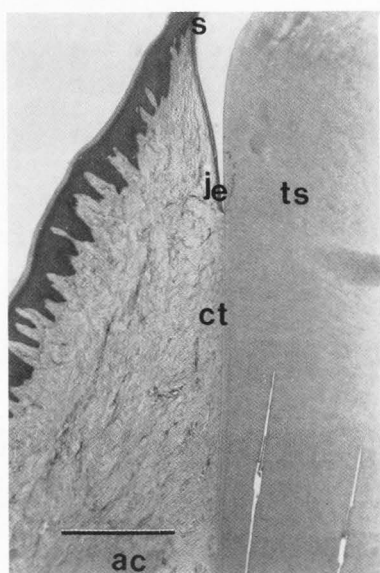


Figure 1. Photomicrograph of an occlusal-apical section of the Dento-Gingival Junction which was obtained by light microscopy. It demonstrates the tooth surface (ts), sulcus (s), junctional epithelium (je), alveolar crest (ac) and the connective tissue attachment (ct). This view is an example of the type of sections used in both light and scanning electron microscopy. Bar = 1 mm.

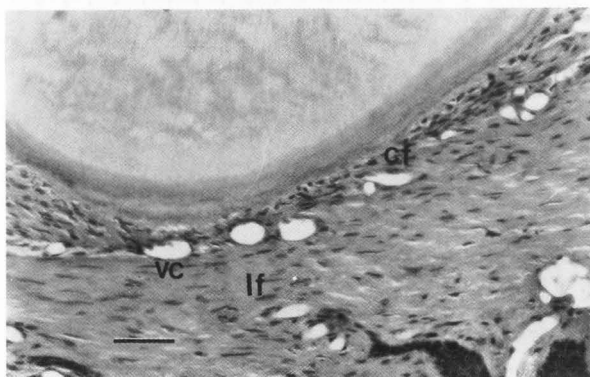


Figure 2. Photograph of a cross-section of the DGJ at the level of the connective tissue attachment. The photomicrograph demonstrates the dense cemental fibers (cf), longitudinal fibers (lf) and vascular channels (vc) near the tooth surface. The cemental fibers extend from the tooth surface and join the longitudinal fibers. Bar = 100 µm.

lingual side of the teeth (Glenwright 1970). Other fiber groups are the transseptal, dentoperiosteal, interpapillary (Melcher 1962) and the intergingival groups (Page et al. 1974). Transseptal fibers extend from tooth to tooth in the interproximal region. All the fibers are interspersed with fibers that are smaller and finer, the reticular-like fibers (Sloan 1978). The periosteum of the alveolar crest, submucosa and lamina propria seem to blend into one firm layer of connective tissue.

The DGJ contains vascular, nerve and lymphatic systems. Vascular terminal loops are seen in the subepithelial papillary layer. The sulcular vascular plexus is seen beneath a junctional epithelium. The sources of the vessels are derived from the periodontium, alveolar bone crest and supraalveolar mucosa. A neural system is present with sensory receptors present in the papillary layer. In the connective tissue adjacent to the base of the sulcus, inflammatory cells are present. These include plasma cells, lymphocytes, neutrophils and macrophages (Attstrom et al. 1975).

Scanning Electron Microscopy

The view of the DGJ as seen with SEM is based on a study of 25 extracted teeth. The health of the gingival tissue varied from normal to slightly inflamed with minimal probing depth. Each patient's treatment plan contained provisions for extraction of teeth, and with their informed consent, the teeth were obtained and processed for SEM. In some specimens adjacent gingival tissue remained attached to the tooth. This was accomplished by the use of vertical incisions on each side of the desired tissue and apical horizontal incisions to free the tissue from the adjacent dental structures, permitting the biopsy to remain on the tooth with minimal distortion during extraction. The specimens were fixed in 2% glutaraldehyde buffered with 0.15M sodium cacodylate, pH 7.2, for at least 24 h. The specimens were then sectioned in the coronal-apical direction maintaining the tooth as thick as possible but exposing the pulp chamber. A groove was placed on the pulpal side of the specimen, opposite of the site to be examined, facilitating the fracture path. Freeze-fracture at the groove was accomplished by rapid immersion of the specimen in liquid Freon 113 that was frozen with liquid nitrogen and slightly thawed. The specimens were then placed in liquid nitrogen until thoroughly frozen and split at the groove with dental extraction forceps. The result was the tissue-tooth interface from the crown toward the apex of the tooth (Figure 3). The specimens were thawed in buffer solution, placed in 70% ethanol for 20 minutes, followed by two

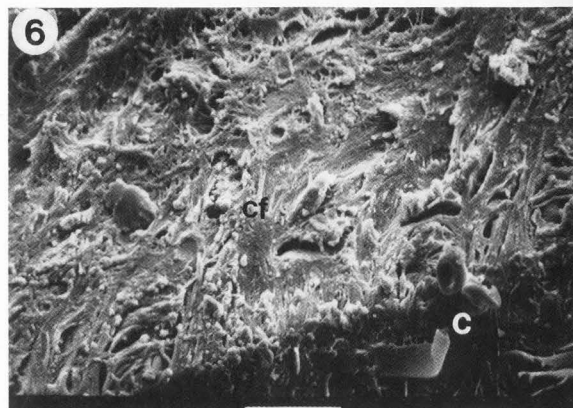
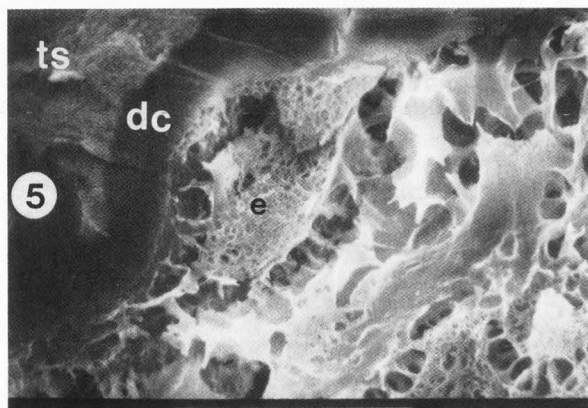
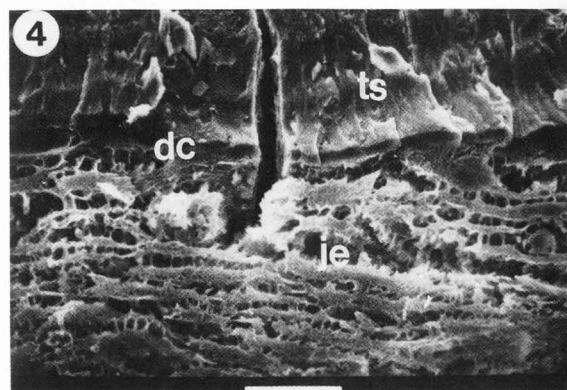
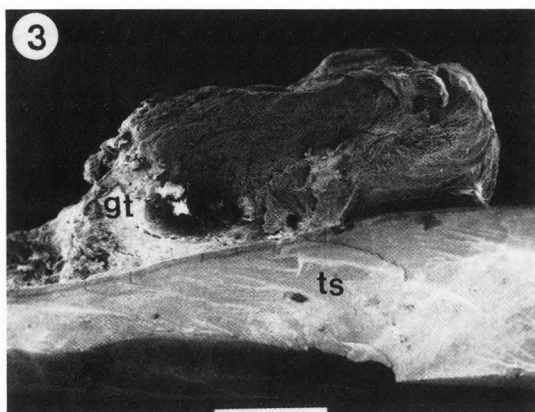


Figure 3. Photograph of a specimen with gingiva attached. This is comparable to the section in Figure 1 viewed with light microscopy. The tooth was freeze-fractured to obtain this view of the DGJ which shows tooth surface (ts) and gingival tissue (gt). Bar = 1000 μ m.

Figure 4. A photomicrograph demonstrating the interface of the tooth surface and junctional epithelium. The human specimen was freeze-dried and coated with gold. The dental cuticle (dc) is located between the junctional epithelium (je) and the tooth surface (ts). Bar = 10 μ m.

Figure 5. Same area as shown in Figure 4 but at higher magnification. The interface demonstrated the presence of the dental cuticle (dc) approximately 2 μ m in thickness between the tooth surface (ts) and epithelial cells (e), Bar = 10 μ m.

Figure 6. Human specimen demonstrating the connective tissue-tooth interface. The specimen was freeze-fractured, CPD, gold coated and viewed with SEM. The photomicrograph demonstrated the dense cemental fibers (cf) forming bundles of different sizes and extending in many directions from the cementum (c). Bar = 10 μ m.

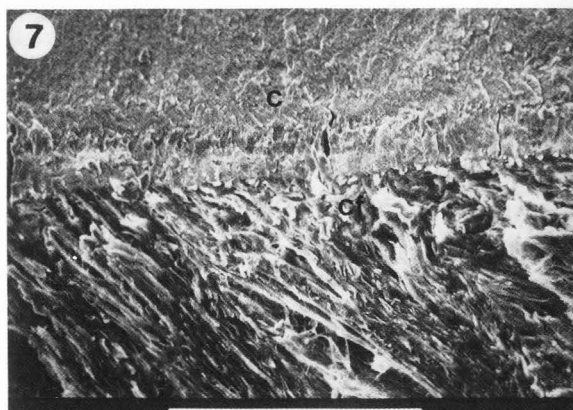


Figure 7. A photograph of the specimen viewed in Figure 4. This view of the connective tissue-tooth interface was derived from a specimen that was freeze-dried. The cemental fibers (cf) are dense and seem to be parallel to each other. The difference with Figure 6 may be due to differences in biopsy techniques, tissue processing, or magnification, c equals cementum. Bar = 100 μ m.

immersions in 100% ethanol. Afterward the specimens were refluxed in ethanol for 24 h, followed by immersion in ethanol-Freon 113 (1:1 ratio), and then immersion in Freon 113. At this point, most specimens were frozen in liquid nitrogen and dried in the chamber of an Edwards-306 evaporation unit (final vacuum of 10^{-5} torr) for 24 h until all Freon was removed. Others were critical point dried from carbon dioxide. After dehydration, the specimens were sputtered with gold (Ernest F. Fullam Inc., Latham, NY) and viewed in an AMRAY-1000A SEM unit.

Observations of the specimens using magnifications not greater than X5000 demonstrated many of the same structures seen with light microscopy in greater detail. A view of junctional epithelium, dental cuticle and tooth surface are seen in Figure 4 and a different view can be seen at higher magnification in Figure 5. These magnifications would not be possible with light microscopy. The dental cuticle was unmineralized (indicated by the use of back scattered electrons), showing a homogeneous, darker structure compared to the tooth and located between the latter and the junctional epithelium. The dental cuticle cannot be observed with light microscopy (Listgarten 1966a,b). Apical to the junctional epithelium, cemental fibers of different sizes, extending directly from the tooth surface and then in different directions (Figure 6). With this fracture technique, the outline of the fibers on the tooth surface may be seen. Also the presence of Sharpey fibers inserting into cementum was observed more clearly than with light microscopy (compare Figures 2 and 6). At X500 magnification (Figure 7) the bundles of fibers appeared to be parallel to each other but at higher magnification (Figure 8), the bundles varied in thickness (approximately 0.5-4 μm) and extended in many directions after leaving the cemental surface. They still were intensely dense at the tooth surface. The differences in direction of bundles could be due to the presence or absence of the gingival tissue mass still attached on the extracted tooth. In addition differences in the appearance of cemental fibers may be caused by tissue processing (compare Figures 6 and 7). Vascular channels were present close to the tooth surface. On the surface of the teeth, fibers were seen extending directly into the lamina propria and other fibers were parallel to and located on the surface (intrinsic fibers) (Figure 8). A large number of fibrils extend between the larger fibers (Figure 9). At intervals near the entrance to the periodontal ligament large bundles which were approximately 90 μm wide and consisting of parallel fibers extended from the surface (Figure 10) and narrowed at 30-40 μm from the surface forming a sheet

of fibers approximately 20 μm wide. Further from the surface of the tooth, various bundles of fibers were seen (Figure 11).

Examination of the sulcus showed different bacterial components or plaque (Figure 12). Supragingival plaque contained cocci with an apparent matrix. Subgingival plaque demonstrated the presence of filamentous, rod, and "corn cob" types (Listgarten et al. 1975). Coronal to the connective tissue attachment on the surface of the extracted tooth, a cell-free zone approximately 100-200 μm wide was present (Figure 13) with connective tissue on the apical and epithelium on the coronal side. The width of this zone was less when compared to the cell-free zone seen in light microscopy (Powell and Garnick 1978).

Discussion

This tutorial paper compares the morphology of the Dento-Gingival Junction as seen with light microscopy and SEM. Each method contributes information useful to the researcher. Light microscopy presents a global picture of the organ, and transmission and scanning electron microscopy illustrates detail that cannot be seen with light microscopy. SEM can view specimens at magnifications similar to light microscopy but can magnify the same areas to much higher levels such as X5000 magnification in this paper. Freeze fracture can be used to obtain views of tissues similar to light microscopy. In addition to high magnification, SEM also enables one to use high depth of field and to image an object's surface (especially freeze-fractured surfaces) instead of cut sections as seen with TEM. Also, using stereoscopic techniques, measurements can be obtained and data can be used to compare results of therapy by statistical evaluation (Boyde 1973, Russ et al. 1986). The material presented in this paper illustrates the benefits of SEM in research of the dento-gingival junction.

The structure of the DGJ is especially important in the pathogenesis and treatment of periodontal disease. The disease process results in the apical migration of the DGJ. In therapy one approach is to extend the attachment of the DGJ coronally. Therefore significant morphological changes, verified by statistical analysis of the measurements from high magnification photomicrographs, can add important information on changes of the DGJ during disease and post therapy.

The epithelial surface covering has been described by others (Cleaton-Jones and Fleisch 1973, Cleaton-Jones 1975, Cleaton-Jones et al. 1978, McMillan 1979, 1980) using the SEM, and is not described in this paper. The sulcular and junctional epithelium have been described in the literature (Kaplan et al.

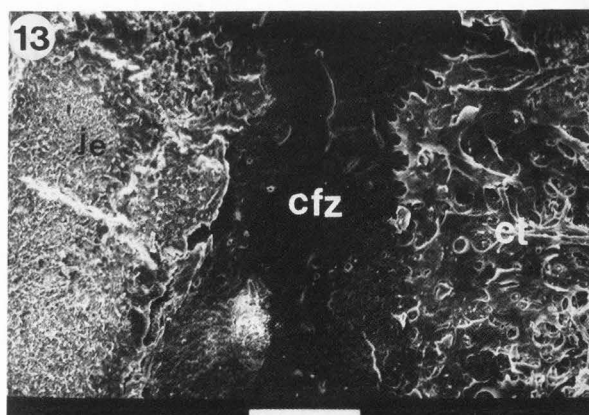
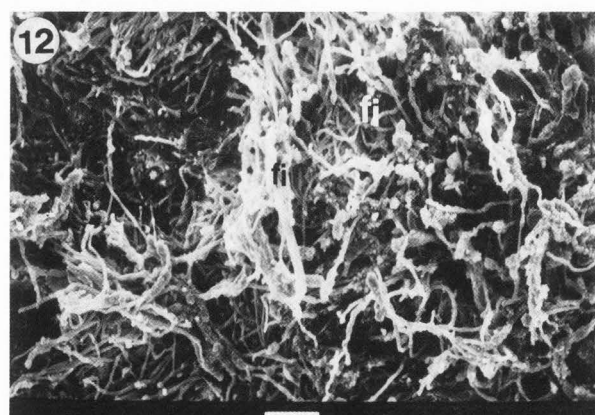
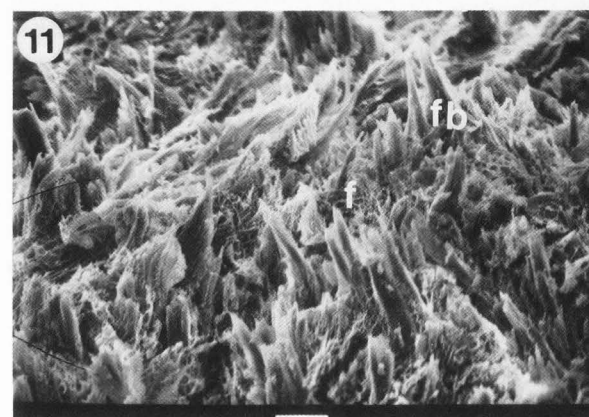
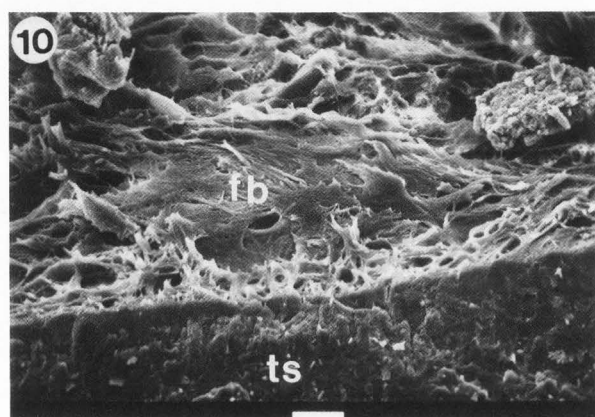
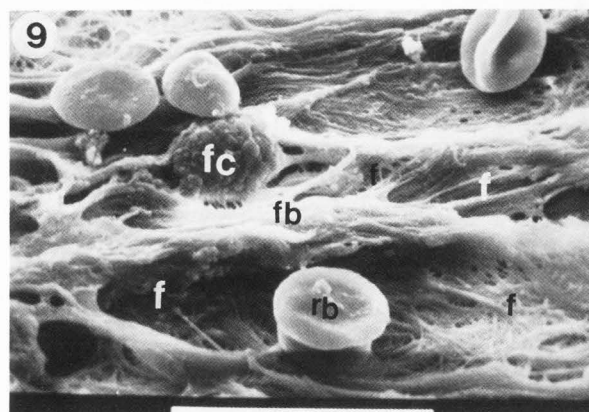
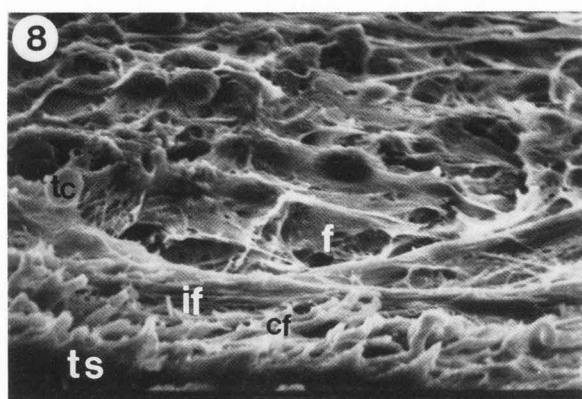


Figure 12 . Human tooth was dehydrated with alcohol, placed in Freon, vacuum dried and coated. The photomicrograph demonstrated subgingival plaque of filamentous type (fi). The contents of the sulcus was considered part of the DGJ. Bar = 10 μ m.

Figure 13 . Same specimen as in Figure 12. The cell-free zone (cfz) was demonstrated and measures approximately 139 μ m between junctional epithelial cells (je) and connective tissue attachment (ct). Bar = 100 μ m.

Figure 8. Specimen of human tooth, freeze-fractured, dehydrated in alcohol, placed in Freon and vacuum-dried. This photomicrograph demonstrated the tooth surface (ts) and collagen fibers (cf) extending from the tooth surface. Also, fibers are parallel to the surface (intrinsic fibers) (if) with fibrils (f) extending between adjacent fibers. Tissue cells (tc) are also present. Bar = 10 μ m.

Figure 9. A photomicrograph of the surface of the tooth seen in Figure 8 demonstrating fibrils (f) extending in many directions between fiber bundles (fb) of the tooth surface. Red blood cells (rb) and perhaps a fibroblast (fc) were present. Bar = 10 μ m.

Figure 10. Photomicrograph from specimen presented in Figure 8 demonstrating a large bundle of fibers (fb) attached to the tooth surface (ts). These bundles were seen toward the apical part of the DGJ. Bar = 10 μ m.

Figure 11. A human tooth-tissue specimen that was freeze-dried and coated. This viewed the lamina propria of the gingiva. Dense collagen fiber bundles (fb) were seen with fibrils running between the larger fibers (f). Bar = 10 μ m.

1977, Kaplan 1977, Saglie 1977, Ooya and Tooya 1981) but not with the use of freeze-fracture techniques to view the interface between the junctional epithelium or cemental fibers and the tooth surface. In this method, the dental cuticle was obvious and the level of connective tissue attachment to the tooth can be easily determined with the use of SEM.

Our paper illustrates the thick attachment of the cemental fibers and the multi-directional extension of these fibers from cementum. The cemental fibers of DGJ have been described using SEM in the literature but again not in the human dentition or with freeze-fracture methods (Shackelford 1971, 1972, Kvam 1972, 1973, Roberts and Chamberlain 1978).

Sloan et al. (1976), Sloan (1978) reported on the importance of tissue preparation in the study of tissue morphology. They reported that collagen bundles of the middle zone of the periodontal ligament formed sheets that extended in the direction of the tooth surface and bone. Fibroblasts were found in the compartments formed by these sheets.

In our view of the lamina propria of the gingiva, collagen fibers formed different size bundles and extended in many directions. The SEM photographs also demonstrated multi-directional small bundles with occasional larger ones extending from cementum. These appeared to be different from fiber bundles in

the periodontal ligament as described by Sloan et al. (1976). The differences may be due to, the different functions of the ligament and the DGJ, and the source of the specimens. The specimens reported here were derived from humans and not animals as described in previously published papers.

SEM has also been used to describe the contents of the sulcus and its relationship to the adjacent structures (Saglie et al. 1974). Our observations using fractured surfaces of teeth illustrated a different type of plaque at the gingival margin compared to that found at the sulcus level. Apical to the sulcus, epithelial cells were present which were possibly part of the junctional epithelium. Apical to the epithelial cells and coronal to the connective tissue attachment was an area on the tooth surface apparently devoid of structures. This zone which was approximately 100-200 μ m wide and referred to by Saglie et al. (1974, 1975b) as the cell-free zone, was not seen in the freeze-fractured specimens. Using SEM methodology, Brady (1973) also described a zone devoid of structures except for a cuticle. This zone can be interpreted as the apical-most area of the junctional epithelium (Bass 1946, 1950). With the use of light microscopy a zone was present between stained plaque and connective tissue attachment that was approximately 0.5 mm wide (Powell and Garnick 1978, Saglie et al. 1975b). A cell-free zone was apparent with the use of SEM that is approximately 0.15 mm wide (Brady 1973, Saglie et al. 1974). The larger width of the zone seen with light microscopy was probably due to low magnification and the inability to determine the presence of cells that were observed at higher magnifications. Based on our observations of freeze-fractured specimens, the cell-free space was not observed apical to the junctional epithelium and the zone contained an homogeneous covering suggestive of a cuticle (Figure 13), (Brady 1973). We interpreted this cell-free area to be covered originally by epithelial cells which were dislodged in obtaining the specimens (Saglie et al. 1974). The specimens presented in this paper represented healthy or slightly clinically inflamed gingiva. A different relationship may be present with advanced periodontal disease (Saglie et al. 1975a).

This paper serves as a demonstration of the effectiveness of SEM in obtaining additional useful views concerning the Dento-Gingival Junction beyond those reported with light microscopy.

References

- Abbas DK, Skjorland KK, Gjermo P, Sonju T (1985). Chemical and morphological studies of the acquired pellicle formed subgingivally on dentin in vivo. *Acta Odont Scand.* **43**:3-37.
- Ainamo J, Loe H (1966). Anatomical characteristics of gingiva. A clinical and microscopic study of the free and attached gingiva. *J. Periodont.* **37**:5-13.
- Anderson GS, Stern IB (1966). The proliferation and migration of the attachment epithelium on the cemental surface of the rat incisor. *Periodontics* **4**:115-123.
- Attstrom R (1970). Presence of leukocytes in crevices of healthy and chronically inflamed gingivae. *J. Periodont. Res.* **5**:42-47.
- Attstrom R, Graf D, Beer M, Schroeder HE (1975). Clinical and histologic characteristics of normal gingiva in dogs. *J. Periodont. Res.* **10**:115-127.
- Bass CC (1946). A demonstrable line on extracted teeth indicating the location of the outer border of the epithelial attachment. *J. Dent. Res.* **25**:401-415.
- Bass CC (1950). Relation of the inner border of subgingival calculus to the zone of disintegrating epithelial attachment cuticle. *Oral Surg. Oral Med. Oral Path.* **3**:1125-1137.
- Bowers GM (1963). A study of the width of attached gingiva. *J. Periodont.* **34**:201-209.
- Boyde A (1973). Quantitative photogrammetric analysis and quantitative stereoscopic analysis of SEM images. *J. Microsc.* **98**:452.
- Brady JM (1973). A plaque-free zone on human teeth - Scanning and transmission electron microscopy. *J. Periodont.* **44**:416-428.
- Cleaton-Jones P (1975). Surface characteristics of cells from different layers of keratinized and non-keratinized oral epithelia. *J. Periodont. Res.* **10**:79-87.
- Cleaton-Jones P, Buskins SA, Volchansky A (1978). Surface ultrastructure of human gingiva. *J. Periodont. Res.* **13**:367-371.
- Cleaton-Jones P, Fleisch L (1973). A comparative study of the surface of keratinized and non-keratinized oral epithelia. *J. Periodont. Res.* **8**:366-370.
- Frithrob L, Wersall J (1965). A highly ordered structure in keratinizing human oral epithelia. *J. Ultrastruct. Res.* **12**:371-379.
- Garant PR, Mulvihill JE (1971a). The ultrastructure of leukocyte emigration through the sulcular epithelium in the beagle dog. *J. Periodont. Res.* **6**:266-277.
- Garant PR, Mulvihill JE (1971b). The ultrastructure of clinically normal sulcular tissues in the beagle dog. *J. Periodont. Res.* **6**:252-265.
- Garguilo AW, Wentz FM, Orban B (1961). Dimensions and relations of the dento gingival junction in humans. *J. Periodont.* **32**:261-267.
- Garnick JJ (1977). Long junctional epithelium: epithelial reattachment in the rat. *J. Periodont.* **48**:722-729.
- Garnick JJ, Walton RF (1984). Fiber system of the facial gingiva. *J. Periodont. Res.* **19**:419-423.
- Gavin JB (1968). The ultrastructure of the crevicular epithelium of cat gingiva. *Am. J. Anat.* **123**:283-296.
- Gavin JB (1972). The effects of histamine on the permeability of gingiva. *New Zealand Dent. J.* **68**:291-299.
- Geisenheimer J, Han SS (1971). A quantitative electron microscopic study of desmosomes and hemidesmosomes in human crevicular epithelium. *J. Periodont.* **42**:396-405.
- Glenwright HD (1970). Observation on circular and longitudinal gingival collagen fibers in the rhesus monkey. *Dent. Pract. (Bristol)* **20**:337-341.
- Goldman, HM (1944). The relationship of the epithelial attachment to the adjacent fibers of the periodontal membrane. *J. Dent. Res.* **23**:177-180.
- Goldman HM (1951). Topography and role of the gingival fibers. *J. Dent. Res.* **30**:331-336.
- Kaplan GB (1977). Scanning electron microscopy of the epithelium of the periodontal pocket - Part II. *J. Periodont.* **48**:634-638.
- Kaplan GB, Pameijer CH, Ruben MP (1977). Scanning electron microscopy of sulcular and junctional epithelia correlated with histology - Part I. *J. Periodont.* **48**:446-451.
- Kobayaski K, Rose GG, Mahan CJ (1976). Ultrastructure of the dento-epithelial junction. *J. Periodont. Res.* **11**:313-330.
- Kobayaski K, Rose GG (1978). Ultrastructure histochemistry of the dento-epithelial junction - Part I - Colloidal thorium and ruthenium red. *J. Periodont. Res.* **13**:164-172.
- Kobayski K, Rose GG (1979). Ultrastructure histochemistry of the dento-epithelial junction. 3-chloramine T-silver methenamine. *J. Periodont. Res.* **14**:123-131.
- Kohl JT, Zander HA (1961). Morphology of interdental gingival tissue. *Oral Surg. Oral Med. Oral Path.* **14**:287-295.
- Kvam E (1972). Scanning electron microscopy of organic structures on the root surface of human teeth. *Scand. J. Dent. Res.* **80**:297-306.
- Kvam, E (1973). Topography of principal fibers. *Scand. J. Dent. Res.* **81**:553-557.
- Lang NP, Loe H (1972). The relationship between the width of keratinized gingiva and gingival health. *J. Periodont.* **43**:623-627.
- Listgarten M (1964). The ultrastructure of human gingival epithelium. *Am. J. of Anat.* **114**:49-64.

- Listgarten MA (1966a). Phase-contrast and electron microscopic study of the junction between reduced enamel epithelium and enamel in unerupted human teeth. *Arch. Oral Biol.* 2:999-1016.
- Listgarten MA (1966b). Electron microscopic study of the gingival-dental junction of man. *Am. J. of Anat.* 119:147-177.
- Listgarten MA (1972). Ultrastructure of the dento-gingival junction after gingivectomy. *J. Periodont. Res.* 7:151-160.
- Listgarten MA, Mayo HE, Tremblay Rollande (1975). Development of dental plaque on epoxy resin crowns in man. *J. Periodont.* 46:10-26.
- Listgarten MA (1976). Structure of surface coatings on teeth. A review. *J. Periodont. Res.* 47:139-147.
- Lopez A, Dooner JJ, Porter K (1976). Histologic study of elastin-like fibers in the attached gingiva. *J. Periodont.* 47:444-449.
- Marikova Z (1983). Ultrastructure of normal and newly formed dento-epithelial junction in rats. *J. Periodont. Res.* 18:459-468.
- McHugh WD (1964). Keratinization of gingival epithelium. *J. Periodont.* 35:338-348.
- McMillan MD (1979). The surface structure of the completely and incompletely orthokeratinized oral epithelium in the rat: a light, scanning, and transmission electron microscopy study. *Amer. J. Anat.* 156:337-351.
- McMillan MD (1980). Transmission and scanning electron microscopic studies on the surface coat of the oral mucosa in the rat. *J. Periodont. Res.* 15:288-296.
- Melcher AH (1962). The interpapillary ligament. *Dent. Pract. and Dent. Rec.* 12:461-462.
- Miyasato M, Crigger M, Egelberg J (1977). Gingival condition in areas of minimal and appreciable width of keratinized gingiva. *J. Clin. Periodont.* 4:200-209.
- Moss JP, Picton DCA (1967). Experimental mesial drift in adult monkeys (*Macaca irus*) *Arch. Oral Biol.* 12:1313-1320.
- Newman HN (1980). Ultrastructural observations on the human pre-eruptive enamel cuticle. *Archs. Oral Biol.* 25:49-57.
- Ooya K, Tooya Y (1981). Scanning electron microscopy of the epithelium-connective tissue interface in human gingiva. *J. Periodont. Res.* 16:135-139.
- Orban B (1948). Clinical and histologic study of the surface characteristic of the gingiva. *Oral Surg., Oral Med., Oral Path.* 1:827-841.
- Page RC, Ammons, WF, Scheetman, LR, Dillingham, LA (1974). Collagen fiber bundles of the normal marginal gingiva in the marsupial. *Arch. Oral Biol.* 19:1039-1043.
- Picton DCA, Moss JP (1980). The effect on approximal drift of altering the horizontal component of biting force in adult monkeys (*Macaca irus*). *Arch. Oral Biol.* 25:45-48.
- Picton DCA (1962). Distortion of jaws during biting. *Arch. Oral Biol.* 7:573-580.
- Porter K, Doaner JJ, Lopez A (1977). Further study of elastic fibers in human attached gingiva. *J. Periodont.* 48:711-713.
- Powell B, Garnick JJ (1978). Evaluation of clinical measurements of periodontal disease. *J. Periodont.* 49:621-624.
- Ramfjord SP, Engler WD, Hiniker JJ (1966). A radioautographic study of healing following simple gingivectomy - Part II - The connective tissue. *J. Periodont.* 37:179-189.
- Roberts WE, Chamberlain JG (1978). Scanning electron microscopy of the cellular elements of rat periodontal ligament. *Arch. Oral Biol.* 23:587-589.
- Russ JC, Hare TM, Christensen RP, Hare KT (1986). SEM low magnification stereoscopic technique for mapping surface contours; application to measurement of volume differences in human teeth due to polishing. *J. Micros.* 144:329-338.
- Saglie R, Johansen JR, Flotra L (1974). Scanning electron microscopic study of tooth surfaces in pathologic pockets. *Scand. J. Dent. Res.* 82:579-583.
- Saglie R, Johansen JR, Flotra L (1975a). The zone of completely and partially destructed periodontal fibers in pathological pockets. *J. Clin. Periodont.* 2:198-202.
- Saglie R, Johansen JR, Tollefsen T (1975b). Plaque-free zones on human teeth in periodontitis. *J. Clin. Period.* 2:190-197.
- Saglie R (1977). A scanning electron microscopic study of the relationship between the most apically located subgingival plaque and the epithelial attachment. *J. Periodont.* 48:105-115.
- Saglie R, Sabag N, Mery C (1979). Ultrastructure of the normal human epithelial attachment. *J. Periodont.* 50:544-550.
- Schroeder HE, Theilade J (1966). Electron microscopy of normal human gingival epithelium. *J. Periodont. Res.* 1:95-119.
- Schroeder HE (1969a). Melanin containing organelles in cells of the human gingiva - Part I - Epithelial melanocytes. *J. Periodont. Res.* 4:1-18.
- Schroeder HE (1969b). Ultrastructure of the junctional epithelium of the human gingiva. *Helva Odont. Acta* 13:65-83.
- Schroeder HE, Munzel-Pedrazzali S (1970). Morphometric analysis comparing junctional and oral epithelium of normal human gingiva. *Helva Odont. Acta* 14:53-66.
- Schroeder HE, Listgarten MA (1971). Fine structure of the developing epithelial attachment of human teeth. A. Wolsky, Editor: *Monographs in Developmental Biology*, Vol. 2, S. Karger: Basel, New York.

Shackelford JM (1971). Scanning electron microscopy of the dog periodontium. *J. Periodont. Res.* 6:45-54.

Shackelford JM (1972). The indifferent fiber plexus and its relationship to principal fibers of the periodontium. *Am. J. Anat.* 131:427-442.

Sims MR (1983). Electron-microscopic affiliations of oxytalan fibers, nerves and the microvascular bed in the mouse periodontal ligament. *Arch. Oral Biol.* 28:1017-1024.

Sloan P, Shellis RP, Berkowitz BKB (1976). Effect of specimen preparation on the appearance of the rat periodontal ligament in the scanning electron microscope. *Arch Oral Biol.* 21:633-635.

Sloan P (1978). Scanning electron microscopy of the collagen fibre architecture of the rabbit incisor periodontium. *Arch. Oral Biol.* 23:567-572.

Smith RG (1982). A longitudinal study into the depth of the clinical gingival sulcus of human canine teeth during and after eruption. *J. Periodont. Res.* 17:427-433.

Smukler H, Dreyer CJ (1968). Principal fibres of the periodontium. *J. Periodont. Res.* 4:19-25.

Stallard RE, Diab MA, Zander HA (1965). The attaching substance between enamel and epithelium - a product of the epithelial cells. *J. Periodont.* 36:130-132.

Taylor AC, Campbell MM (1972). Reattachment of gingival epithelium to the tooth. *J. Periodont.* 43:281-293.

Tenenbaum H, Tenenbaum M (1986). A clinical study of the width of the attached gingiva in the deciduous, transitional and permanent dentitions. *J. Clin. Periodont.* 13:270-275.

Voigt JP, Garan ML, Fleister RM (1978). The width of lingual mandibular attached gingiva. *J. Periodont.* 49:77-80.

Waerhaug J (1956). Enamel cuticle. *J. Dent. Res.* 35:313-322.

Walsh LJ, Seymour GJ, Powell RN (1986). Interleukin-1 modulates to expression on a putative intra-epithelial Langerhans cell precursor population. *J. Dent. Res.* 65:1424-1426.

Weiss L, Neiders ME (1970). A biophysical approach to epithelial cell interactions with teeth. *Adv. in Oral Biol.* 4:179-260.

Wertheimer FW, Fullmer HM (1962). Morphologic and histochemical observations on the human dental cuticle. *J. Periodont.* 3:29-39.

Yamaski A, Nikae H, Nutani K, Ijuhin N (1979). Ultrastructure of the junctional epithelium of germfree rat gingiva. *J. Periodont.* 50:641-648.

Discussion With Reviewers

R. Saglie: Could you explain the advantages of using LM and SEM as separate tools in your study compared to (a) LM and SEM as a combined instrument or (b) correlative LM and SEM?

Authors: We were interested in the tissue-tooth interface obtained by freeze-fracture. Using the combined technique, the processing of specimens must include embedding the tissue and therefore limits the advantages of freeze-fracture. Correlative methods have advantages if performed after the freeze-fracture process. One portion would be used for SEM and the other for LM.

A. Carrassi: With this technique of freeze fracture, can spontaneous and regular fracture of the soft and hard tissue also occur without application of a dental forceps?

Authors: It was our experience that spontaneous fracture rarely occurred; thus, the forceps were needed to initiate the fracture.

A. Carrassi: In the sample studied with SEM, clarify the reason for extraction and the degree of periodontal involvement.

Authors: The teeth were obtained during multiple extraction procedures. Adjacent teeth were extracted because of extensive carious lesions and/or periodontal disease and the sample teeth were included as part of the overall treatment plan. The gingiva were considered healthy or had mild gingival inflammation with limited probing depth. Mild inflammation had to be an acceptable criteria to obtain a sufficient number of specimens.

A. Carrassi: How did the authors distinguish between the dental cuticle and the epithelial attachment with back scattered signals?

Authors: The cuticle as seen in this report, appeared as a homogeneous darker band between the tooth surface and the cell membrane of the adjacent epithelia. This band may contain contributions of the attachment apparatus of epithelial cells. However in the view with BSE, the shape and homogenous appearance of the dental cuticle contrasted with the irregularities and shape of cell membrane.